Method and device to detect therapeutic protein immunogenicity

Stephen E. Zweig, Los Gatos, California

Background of the Invention

This application claims the priority benefit of provisional patent application 60/465,434, "Electronic time-temperature indicator", filed April 25, 2003, provisional patent 60/496,358 "Method and device to reduce therapeutic protein immunogenicity", filed August 18, 2003, and copending patent 10/634,297 "Electronic time-temperature indicator", filed August 5, 2003.

Field of the Invention

This patent application covers methods and devices by which unwanted immune responses against therapeutic proteins may be detected and prevented.

Description of the related art

Recent advances in genetic engineering and biotechnology have enabled the creation of a number of advanced biotherapeutic drugs, which are usually therapeutic proteins produced by recombinant DNA techniques. These drugs, such as recombinant insulin, interferon, erythropoietin, growth hormone, and the like, have revolutionized modern medicine.

One thing that most modern biotherapeutic drugs have in common is that they often are recombinant DNA cloned versions of natural proteins and protein hormones, or are modified versions of natural proteins. As such, most biotherapeutics have a much higher molecular weight than traditional pharmaceuticals. Additionally, most biotherapeutics tend to be somewhat delicate. Whereas most traditional pharmaceuticals are small

molecules, typically robust and resistant to deterioration caused by temperature storage effects, this is not the case for therapeutic proteins. Many biotherapeutic drugs are dependent upon the correct conformation of their protein components. As a result, biotherapeutics are quite temperature sensitive. Many cannot tolerate freezing, because freezing tends to denature proteins and cause the formation of protein aggregates. Many also cannot tolerate storage temperatures much above refrigerator temperatures, since higher temperatures can also promote protein denaturation and formation of protein aggregates. As a result, most modern biotherapeutics must be carefully temperature controlled from the time of manufacture, to the time they are used by the ultimate end user.

The immune system is a complex network of immune system cells, antibodies, cytokines, and other regulatory components designed to detect and destroy foreign (non-self) molecules, while at the same time not attacking native (self) molecules. Thus molecules that naturally occur in the body exhibit immune tolerance. The biological reason for this should be clear, since it is obviously undesirable for the body to attack its own naturally occurring components. Biotherapeutics, by virtue of the fact that they are synthetic analogs of naturally occurring proteins, also are often covered by this same immune tolerance system. Thus medical practice typically assumes that a biotherapeutic that is an analog of a naturally occurring molecule should generally be capable of administration without undue concern for provoking an immune reaction. However as the structure of a biotherapeutic molecule diverges from a native molecule, the possibility of it triggering a "foreign molecule - attack" immune response increases. In particular, the immune system often recognizes protein aggregates as "non-self", and mounts an immune response against them. Such targets of immune system attack are commonly referred to as "antigens".

Although modern biotherapeutics have saved countless thousands of lives, and improved the quality of life for countless others, as their use has increased, it has become apparent that the drugs occasionally exhibit unwanted side effects. One of the most distressing side effects is the occasional development of an unwanted immune reaction against the

biotherapeutic. This effect is discussed in Rosenberg, Immunogenicity of Biological Therapeutics, A Hierarchy of Concerns, Dev. Biol. Basel, Karger 2003, Vol 112, pp15-21. These unwanted reactions are sometimes referred to as HADA (human anti-drug antibody) effects.

As discussed in Chamberlain, "Immunogenicity of Therapeutic Proteins", The Regulatory Review 5:5, August 2002,, pp 4-9 , such unwanted immune responses can range from mild responses, to very severe responses. In the mild case, which often occurs for diabetics exposed to partially degraded insulin delivered by insulin pumps, antibodies against the biotherapeutic partially neutralize the biotherapeutic, requiring the dose of the biotherapeutic to be increased in order to achieve the same therapeutic effect. Thus in this insulin pump example, affected diabetics require increasingly larger doses of recombinant human insulin in order to achieve good blood glucose control. In other cases, such as has been seen with recombinant erythropoietin (which is a recombinant protein analog to a naturally produced red cell production stimulating hormone), more serious effects can occur. Erythropoietin is often used to stimulate red blood cell production in anemic patients. However antibodies induced by the recombinant erythropoietin biotherapeutic can bind to naturally produced erythropoietin. This can lead to the complete cessation of all subsequent red cell production. This later condition, called "red cell aplasia" can be fatal unless treated by blood transfusion and/or immunosuppressive drugs.

Although vibration, shaking, or light exposure can facilitate the degradation of therapeutic proteins, these effects are usually minor, relative to temperature effects.

It is generally recognized that upon storage, therapeutic proteins degrade by a variety of time- temperature dependent processes, including denaturation, aggregation, oxidation, deamidation, disulfide exchange, and proteolysis. Studies have shown that this time and temperature dependent storage degradation can create immunogenic byproducts, such as protein aggregates, and further have shown that the formation of these immunogenic byproducts is accelerated at higher storage temperatures (Hochuli, "Interferon

Immunogenicity: Technical Evaluation of Interferon α2α", J. Interferon and Cytokine Res. 17 supplement 1: S15-S21, 1997).

Although storing therapeutic proteins at a lower temperature can minimize a number of these processes, other temperature effects often impose a practical lower temperature storage limit. Upon freezing, for example, many proteins undergo conformational changes that can also lead to denaturation, and aggregation. Thus in practice, therapeutic proteins are optimally stored in a rather narrow temperature range, typically 2-8 °C.

Curiously, although it is well known that therapeutic proteins are very sensitive to the effects of time and temperature on storage, in general, the biotechnology and pharmaceutical industry has exhibited a profound lack of curiosity as to the effect on biological therapeutics of storage at temperatures other than refrigerated temperature (2-8 °C), room temperature (generally 23-25 °C), or mild elevated temperature (30 °C). There are very few published studies discussing stability outside of these few specified temperature conditions. This lack of curiosity may be due, in part, to the pharmaceutical industry's tradition of working with small molecule drugs, which are typically less temperature sensitive, less immunogenic, and which usually exhibit tolerance to a broad range of storage conditions. In general, the unstated assumption for biotherapeutics has been that it is adequate to simply characterize a therapeutic protein's temperature stability at a few points, and assume that the therapeutic protein will never encounter any other type of temperature conditions after initial shipment.

At present, when pharmaceutical products are shipped, it is standard practice to include temperature monitors as shipping indicators. These monitors, such as the HOBO time-temperature data logger produced by Onset Computer Corporation, Pocasset, MA; the Monitor In-transit temperature recorder; the TagAlert® and TempTales® monitors, produced by Sensitech Corporation, Beverly Massachusetts; and others; inform users if the drug has been exposed to temperature extremes during shipment. However after shipment, such monitors are typically removed.

Similarly, it is common practice to store drugs in refrigerators, which when run in a properly managed health care practitioner setting, will also be monitored and controlled. Normally, however, drugs are stored in more than one refrigerator during their storage lifetime, and this is where problems can occur.

Note that at present, the cold chain between the manufacturer and the ultimate end user has many interface boundaries. At these boundaries, time-temperature monitoring by one system ends, and monitoring by a different system begins. The time and temperature conditions in the boundary between these different systems is usually not monitored or tracked.

Clearly, it is unrealistic to assume that in all steps and interface regions of the cold chain between the pharmaceutical manufacturer and the ultimate use by the health care practitioner or patient, all protein therapeutics will always be carefully temperature controlled. Other areas of medicine do not make such optimistic assumptions. In medical diagnostics, for example, manufacturers and regulators assume that recommended storage and handling conditions may, in fact, be violated. As a result, diagnostics manufacturers and regulators often require that medical diagnostic products incorporate one or more controls or detection methodologies to detect if the diagnostic's recommended storage and handling conditions have been violated. Such approaches are taught by US patent 6,629,057, and other technology. In this respect, the disparity of practice between the medical diagnostics industry, and the biotherapeutic industry, is quite large.

One explanation for the difference in practice between the medical diagnostics industry and the biotherapeutic industry is ease of quantitation. Medical diagnostics are designed to rapidly convey large quantities of precise numeric information as to their operating condition. Thus problems can be quickly and easily detected. By contrast, biotherapeutics are more difficult to assay, and immunogenicity assays are particularly difficult. However given the now large number of cases in which immunological

complications of protein biotherapeutics have been reported, it is clear that these issues need to be addressed.

Consider, for example, the consequences of improper storage conditions on three different products: the first is a food product, the second is a medical diagnostic, and the third is a biotherapeutic protein. In the first case, customers will quickly detect food degradation, either through "off" taste, or possibly food sickness, and the improper storage will be quickly discovered and corrected. In the second case of a medical diagnostic product, the improper storage will also be quickly detected when lab operators run controls, and obtain aberrant answers. Here too, improper storage will be quickly discovered and corrected. However in the third case of a therapeutic protein, the results may be quite different. On a somewhat random basis that may correlate with shipment or storage history, but which will usually not correlate with specific manufacturing lot numbers, certain patients may develop inexplicable immune reactions against the therapeutic protein. This will typically occur many months after the fact. Given the large time lag, difficulty of detection, and the random nature of improper storage conditions, the cause may never be discovered. Yet at the same time, the consequences may be severe. A therapeutic protein pharmaceutical product, or indeed an entire class of therapeutic protein pharmaceuticals, may be subject to regulatory delay or outright recall, affecting the medical status and prognosis of thousands of patients worldwide.

Whether a potentially antigenic therapeutic protein proceeds to produce a clinically unacceptable immune response in a patient depends upon a number of additional factors. Patients differ in their genetic makeup, with some patients tending to be antigen "responders", and some tending to be antigen "non responders". Additionally, the route of administration of the antigen may play a role. Mounting an immune response generally takes time. Therapeutic proteins administered in a localized depot, such as by subcutaneous injection, which slowly produces a higher localized level of antigen, may produce a higher immune response than therapeutic proteins administered by an intravenous route. Although differences in patient genetic makeup and route of administration will clearly have an impact on the development of an unacceptable

immune response, clearly a key strategy is to simply avoid using potentially antigenic therapeutics in the first place.

Currently, the biotechnology industry expends a great amount of effort in optimizing the chemistry of biotherapeutics, with the goal of minimizing immunogenicity. These efforts include humanizing monoclonal antibodies, modifying the structure of the biotherapeutic proteins, and optimizing the pH, buffer, and carrier molecules that help preserve the original biotherapeutic shape and structure. However in contrast to this extensive amount of effort to optimize biotherapeutic chemistry, a relatively small amount of effort is devoted to monitoring the storage conditions that can cause chemical modifications and antigen formation upon prolonged biotherapeutic storage.

In medical diagnostics, and in many other areas, causes of failure are often analyzed by FMEA (Failure Modes Effects Analysis). This type of analysis allows failure modes to be numerically ranked in order of importance, based upon the severity of the failure, the frequency of occurrence of the failure, and the ability to detect the failure in a timely manner. More severe failures are given a high numeric first coefficient, more frequent failures are given a high numeric second coefficient, and hard to detect failures are given a high numeric third coefficient. Easy to detect failures are generally given a low numeric rating, since failures that can be easily detected can then usually be counteracted quickly. The three coefficients are then multiplied, and the magnitude of the resulting FMEA rating is used as a guide to determine the order and priority in which failure modes should be addressed. Higher FMEA ratings are more urgent, and are generally given a higher priority for subsequent corrective action.

FMEA analysis can be used to examine the three examples of improper storage conditions discussed previously. The first example, improper food storage, although important, would generally be given a medium FMEA priority because the failure is usually simply customer dissatisfaction or gastric distress, and the ability to detect the failure is high. Improper medical diagnostics storage might be given a somewhat higher priority, due to the fact that the impact severity, possible misdiagnosis of a patient, is

often quite high. However since control tests are mandated, and frequently performed, the detectability is also high, and the good detectability FMEA coefficient reduces the overall FMEA ranking. By contrast, improper shipment or storage of a protein therapeutic will typically generate a very high FMEA score. The failure mode, possible patient adverse reaction to the drug, possible death, and possible recall of an otherwise promising therapeutic, is extremely severe. At the same time, using current practice, a number of storage condition failures are often difficult or impossible to detect, due to lack of appropriate devices to continually monitor the material at all steps of the cold chain. This combination of high impact and low detectability is quite undesirable. As the frequency of such events increases, the subsequent FMEA ranking may get very high.

At present, pharmaceutical manufacturers are primarily focused on reducing the severity and frequency portion of the FMEA analysis by employing chemical strategies intended to reduce the potential antigenicity of the therapeutic proteins. Although this effort is justified and commendable, FMEA analysis shows that there is another way to reduce risk. This is by improving the detectability of the failure. Health care practitioners or patients who are aware that a particular vial of therapeutic protein has a potential immunogenicity issue due to improper storage or handling can simply avoid using that particular vial. This can be done by incorporating monitoring means with the vial that stay with the vial throughout the cold chain, and that can warn the user about potential immunogenicity issues. Although traditionally, limitations in sensor technology have made such efforts technically or economically infeasible, the rapid advance in modern low cost electronics, instrumentation and detection chemistry, as well as the comparatively high economic value of each vial of therapeutic protein, now make such efforts feasible.

Summary of the Invention

The present invention consists of a time-temperature indicator device that has at least one parameter set to warn when a therapeutic protein drug has had a thermal history

associated with increased risk of unwanted immunological activity. The indicator device is designed to remain with the drug as the drug travels throughout different links of the cold chain. In a preferred embodiment, the indicator device remains associated with the therapeutic protein from the time of manufacture up until the final few minutes before the drug is used. In alternate forms of the invention, additional parameters, including motion, light, and turbidity may also be monitored. Novel methods for determining therapeutic protein time-temperature immunological risk parameters, and programming or adjusting the indicator device, are also disclosed.

At least one of the parameters of the time-temperature indicator devices of the present invention is determined by tests for immunological stability, which is distinct from functional stability. The final stability of the therapeutic protein is determined based on a function that incorporates both the time and temperature profile required to maintain functional activity, and the time and temperature profile necessary to avoid the production of therapeutic protein degradation products that are typically associated with risk of unwanted immunological activity.

Since the immune system is extremely sensitive, only a small amount of degradation, on the order of a few percent or less of the total material, may trigger an unwanted immune response. Thus often, such degraded material, although now immunologically unacceptable, may otherwise still perform adequately in all other therapeutic areas. For example, a therapeutic protein may lose from < 1% to 10% of its protein to a degraded and potentially antigenic form, yet not show any significant change in functional activity, since 90 to 99% of the material would still be unaffected. Thus typically the immunological stability of a therapeutic protein is affected before the functional stability of the protein is affected. That is, a protein tested and released to strict immunological stability standards will typically have a restricted time and temperature stability profile, relative to proteins tested and classified only by standard (and non-immunological) functional stability criteria.

Such indicators could be particularly useful for biogenerics. Biogenerics are therapeutic proteins that have gone "off patent", and are now produced by alternate manufacturers as generic drugs. Such biogenerics are often produced by methods that are slightly different from the original proprietary form of the therapeutic protein. Given the complexity of large molecular weight proteins, there is a potential risk that the new manufacturing processes will produce products may, upon temperature stress, degrade into material that creates an immunological risk. Such risks can be mitigated by carefully characterizing the environmental conditions likely to produce antigenic protein degradation products, and programming this data into indicator devices that can remain associated with the biotherapeutic throughout its product life.

Brief description of the drawings

Figure 1 shows a population of therapeutic proteins before and after thermal stress.

Figure 2 shows a hypothetical stability profile for a therapeutic protein.

Figure 3 shows a programmable time-temperature indicator.

Figure 4 shows the stability lifetime of Eprex™ and Neorecormon™ forms of erythropoietin.

Figure 5 shows a graph of the coefficients of a time-temperature program designed to mimic the observed functional and immunological stability of Eprex and Neorecormon.

Figure 6 shows a unitized container - environmental sensor for a therapeutic protein.

Figure 7 shows a unitized programmable electronic time-temperature indicator.

Figure 8 shows a pharmaceutical container containing an electronic time-temperature indicator.

Detailed description of the invention

Although the concept of monitoring storage containers of therapeutic agents is not new, in the past, such monitoring has been focused entirely on detecting loss of therapeutic activity, rather than in detecting formation of unwanted immunogenic activity.

Prior examples of monitored therapeutic agents include HeatMarker® Time-Temperature indicator (LifeLines Technology, Morris Planes, New Jersey) labeled vaccine vials. These are useful for distributing vaccines in third world countries, where vaccines may become inactive (loose their immunogenic potential) due to exposure to high temperatures for too long a time. Here, the indicator device is a temperature sensitive label stuck to the outside of a vaccine vial. The label changes color in response to exposure to high temperatures for too long a time, and thus warns the user if the vaccine has degraded (lost immunological activity).

These previous combination therapeutic agent containers - environmental detector systems differ from the present invention in that, for the case of vaccines, antigenic activity is an essential component of the therapeutic. Here the detectors are designed to detect temperature-induced loss of antigenic activity. By contrast, the present invention is designed for therapeutic agents that are not normally antigenic, and indeed where antigenic activity is unwanted. An additional difference is that the prior art indicators, being chemically mediated, typically were insensitive to freezing conditions, where proteins frequently denature and start to exhibit antigenic activity.

The present invention has two aspects. The first aspect of the invention is based upon the concept of using "immunological stability" as one of the primary criteria for determining the shelf life and storage conditions of therapeutic proteins, and using this data as a key

input into the final assessment of the therapeutic's final "acceptable stability" profile. Here, the utility of using immunological stability for shelf life dating is proposed, along with various methods to determine immunological stability shelf life and storage conditions.

In the second aspect of the invention, indicator devices are disclosed that continually monitor a therapeutic protein's storage conditions, and warn users when the immunological stability profile of the therapeutic has been exceeded, and can also warn when other time-temperature storage criteria have been exceeded.

As previously discussed, as a therapeutic protein degrades, often antigenic activity may develop before the extent of degradation is large enough to produce a significant change in the therapeutic efficacy of the protein. This is because, for example, a protein changing from a 100% monomeric state to a 95% monomeric, 5% aggregated state will typically suffer, at most, only a 5% loss in potency, which is generally too small to be observable. By contrast, the concentration of the potentially antigenic aggregates will have changed from 0% to 5% of the total amount of therapeutic protein, which is essentially an infinite increase. As a result, antigenic degradation limits will often impose more stringent time and temperature limits on a therapeutic protein's lifetime then will potency loss limits.

To avoid unwanted side effects due to antigenic activity, more stringent "antigenic generation" criteria should be used to determine the storage stability of biological therapeutics.

Figure 1 shows a diagram of some of the fundamental biochemistry and immunology behind the present invention. That is the difference between a therapeutic protein's functional stability, and a therapeutic protein's immunological stability.

Figure 1 shows some of the mechanisms by which a therapeutic protein can deteriorate as a result of suboptimal storage conditions (excess temperature for too long a time,

freezing, etc.). When freshly manufactured, therapeutic proteins typically exist as a homogenous population of non-aggregated, active, molecules (1). Upon suboptimal temperature storage or other adverse conditions (2), this homogeneous population of molecules can undergo a number of different degradation reactions. In the degraded population (3), many of the therapeutic protein molecules retain their original conformation, and activity. Thus from a functional standpoint, this degraded population may contain enough functional therapeutic proteins (4) so as to retain normal functional activity. From a functional stability standpoint, population (3) is still acceptable.

However from an immunological stability standpoint, the situation may be different. Figure 1 shows two possible degradation modes. One harmless degradation mode, shown in (5) may produce degraded proteins that may or may not have degraded functional activity, but are not inherently more antigenic, or prone to stimulate unwanted immunological reactions.

Figure 1 also shows a second harmful degradation reaction that produces immunogenic protein aggregates (6). These protein aggregates may, or may not, have degraded functional activity, and may be undetectable in a functional assay. However as the concentration of protein aggregates increases (6), the chances for an undesired immunological reaction also increase.

Figure 2 shows a graph showing the rate of deterioration of a hypothetical therapeutic protein at various temperatures. Figure 2 (1) (line 1) shows the rate of deterioration of the functional activity of the protein. Typically this deterioration is due to the sum of all degradation processes that operate upon the protein, and the amount of deterioration only becomes large when the sum of all degradation processes significantly reduces the total concentration of active therapeutic protein.

Figure 2 (2) (line 2) shows the rate of formation of immunologically active deteriorated protein components. Typically, only a very small amount of immunologically active deteriorated protein needs to be produced to create immunologic (HADA) stability issues.

Additionally, only some of the deteriorated protein products, such as formation of aggregates, may be responsible for unwanted immunological activity. As a result, line 2 often, but not always, may show greater temperature sensitivity than line 1. In this diagram, the effective optimal stability temperature from the standpoint of functionality is shown as (3), and the effective optimal stability temperature from the standpoint of immunological activity is shown as (4).

In the case where the immunological activity time-temperature range is broader (more robust) than the functional activity time-temperature range, no adjustment in therapeutic protein stability time temperature lifetime criteria is needed because the functional time-temperature stability profile are conservative, and protect patients from unwanted immunological activity. However in the more frequent case where the immunological activity time-temperature range is narrower (less robust) than the functional activity time temperature range (illustrated in figure 1), then to avoid potential unwanted immunological side effects, the time-temperature stability profile of the therapeutic protein should be revised.

Methods to monitor the immunological stability of therapeutic proteins

In certain cases, immunological stability considerations may cause the time-temperature storage characteristics of a therapeutic protein to be substantially derated, relative to its nominal functional stability profile. Although occasionally, a simple labeling change, in which a therapeutic is simply given a more conservative set of storage temperatures and storage lifetime, will be sufficient way to address these issues, often this will not be enough. In order to provide a robust solution that is capable of coping with the inevitable disruptions in the cold chain that will occur with large-scale commercial distribution, (discussed in the earlier FMEA analysis) it will often be desirable to incorporate active time-temperature monitoring means into the therapeutic protein's storage container.

As a less favored embodiment of the present invention, chemistry based integrating time-temperature indicators may be used. For example, the LifeLines HeatMarker® (Baughman et. al. US patent 4,389,217, Prusik et. al. US patent 6,544,925) or 3M MonitorMark® (Arens et. al. US patent 5,667,303) colorimetric time-temperature monitors may be used. However since therapeutic proteins are typically subject to deterioration at both low and high thermal conditions, standard chemical time-temperature indicators, which typically only trigger on higher temperatures, and may not precisely model the exact characteristics of the therapeutic drug, may not be adequate for all situations.

A more favored embodiment of the present invention is based upon the improved electronic time temperature indicators disclosed in copending US patent application 10/634,297, "Electronic time-temperature indicator", filed August 5, 2003, and incorporated herein by reference. These electronic time-temperature indicators can be made to be highly accurate, and customized to address nearly any conceivable set of time-temperature algorithmic criteria. Other electronic time-temperature monitors, such as those disclosed in Berrian et. al., (US patent 5,313,848; and subsequently reexamined and reissued as Re. 36,200), may also be used, whenever the immunological and chemical parameters of the biotherapeutic in question allows the less flexible time-temperature performance of this earlier technology to be used.

Although non-indicating time-temperature indicators, such as radio frequency identification (RFID) tag time-temperature indicators, such as the Bioett RFID tag (Sjoholm et. al. WIPO application WO0125472A1), or electronically communicating temperature loggers, such as the Dallas Semiconductor iButton (Curry et. al. US patent 6,217,213) may also be used, these are generally less preferred, because these systems lack visual displays capable of giving immediate feedback to healthcare practitioners and/or patients.

Figure 3 shows an electrical schematic of a preferred time-temperature indicator, constructed according to the teaching of copending application 10/634,297, that is well

suited for use in the present invention. This has a microprocessor or microcontroller (1) receiving thermal input data from a temperature sensor, such as a thermocouple or thermistor (2). The microprocessor (1) further receives algorithms from stability memory (3) containing instructions for converting the thermal data into numeric data proportional to the stability impact of the measured temperature upon the monitored material. Microprocessor (1) will typically contain an onboard timer, as well as other general programming information in its own onboard memory.

Microprocessor (1) will have at least one output means. Usually this output means will be a visual output means, such as a liquid crystal display ("LCD") (4). Other output means, such as LEDs, sonic alarms, vibration, radio frequency signals, electrical signals, and infrared signals may also be used. This output means, here exemplified by a liquid crystal display, will at a minimum be able to convey to the user the information that the stability characteristics of the unit have been determined to be acceptable (here designated by a "+" symbol), or non-acceptable (here designated by a "-" symbol).

Although other power sources are possible, microprocessor (1), and other power consuming circuitry in the unit, will typically be powered by battery (5). An example of such a battery is a 1.5 Volt or 3 Volt coin cell.

The microprocessor may optionally have manufacturer input means, such as a reset button (6) that zeros and reinitializes the unit. The microprocessor may also optionally have a second user input means, such as a test button (7), that may instruct the unit to transmit supplemental temperature statistical data.

In order to make the time-temperature unit as versatile as possible, the processor memory containing the material stability data (3) may be designed to be a rewriteable memory, such as an electrically erasable programmed read only memory (EEPROM), or flash memory. This EEPROM or flash memory may be reprogrammed by signals from a programming device external to the unit (8). Alternatively, the stability data may be on a replaceable chip (such as a memory card chip), or other memory storage device, which is

plugged into the unit, or be an integral part of the microprocessor's own nonvolatile memory.

It is generally convenient to place all the circuitry, including the battery, processor, thermistor (temperature sensor), buttons, and display into a unitized case (9), so as to present a single device or unit to the user. This device may optionally have attachment means, such as adhesive, Velcro, hooks, snaps, etc., to enable the device to be attached to the vial or container holding the therapeutic proteins. If data output is desired, optional infrared, electrical, or radio frequency port (10) may be used to output relevant temperature statistics and other verification data upon pressing of the test button (7).

Typically, to allow more precise monitoring of the therapeutic protein's temperature, the thermocouple or temperature sensor (2) may be embedded into the case wall, or mounted outside of the case. These later configurations may be preferred for situations where the monitor will be stuck directly onto the material to be monitored. In a fourth configuration, temperature sensor (2) may be mounted in the hole or junction between the case and the inside of the therapeutic protein package, and be directly exposed to the interior of the package, gaining some physical protection while minimizing thermal interference from the case wall itself.

As previously discussed, to allow this device to be rapidly customized for a particular therapeutic protein, it is advantageous that the stability lookup table or conversion function data be stored in a non-volatile read-write storage medium, such as Electrically Erasable Programmable Memory (EEPROM), flash memory, or equivalent. However if this convenience is not desired, and cost minimization is priority, a non-reusable memory, such as a programmed read only memory (PROM), or read only memory (ROM) may also be used.

In some embodiments, the stability data stored in (3) may be in the form of a lookup table. In alternate embodiments, the data may be stored in the form of a mathematical function that automatically generates the equivalent information.

Microprocessors suitable for the present invention are typically ultra low power microprocessors, with a corresponding long battery life. These microprocessors may additionally incorporate a number of onboard functions such as timers, liquid crystal display drivers, analog to digital converters, and circuitry to drive temperature sensors. The MSP430 family of microprocessors, such as the MSP430F412, produced by Texas Instruments, Inc., exemplifies one such microprocessor type. This processor family includes members with onboard reprogrammable flash memory, as well as analog to digital ("A/D") converters, timers, LCD drivers, reference current sources to power sensors, and other functions. Here, the stability data may be directly downloaded into the flash memory on the same chip that holds the other processor components.

Other types of time temperature monitor, or other environmental monitor, may also be used. As one example, if the therapeutic protein is sensitive to vibration or motion, the monitor may also have motion-sensing means. If the therapeutic protein is sensitive to light, the monitor may also have light sensing means. If the therapeutic protein forms turbidity in response to environmentally induced damage, light scattering sensing means may also be used. Typically the monitor will have at least an ability to monitor both time and a function of temperature, so as to adequately warn if the effects of temperature over time on the therapeutic protein are leading to the formation of undesirable immunological byproducts.

Methods to determine onset of immunogenicity:

Although the simplest and most direct method to determine the time-temperature degradation threshold where therapeutic proteins become antigenic is by experimental injection and immune response detection, such methods are usually infeasible.

In the direct approach, samples of the therapeutic protein are stressed to a varying extent, and used to immunize experimental subjects. Although humans are the most realistic subjects, this is legally and ethically impermissible, and thus experimental alternatives

must rely upon model animals such as mice, which many not accurately reflect the immune response of a human population.

Thus due to the complexity of the immune response, and the infeasibility of working with the large numbers of human subjects required to get a definitive assessment, typically more indirect immunological risk assessment methods must be used.

At present, immunogenicity risk is primarily assessed by indirect methods, which monitor the physical degradation or change in the protein, and attempt to assess when such changes are likely to trigger an immunological reaction.

In general, aggregated proteins tend to be more immunogenic than non-aggregated proteins, and the progressive development of protein aggregates is a good marker for potential immunogenic activity. Thus one of the simplest immunogenic reactivity methods is to monitor the time-temperature storage conditions that promote the formation of larger molecular weight protein aggregates.

Methods to monitor protein aggregate formation include light scattering, size exclusion chromatography, centrifugation, mass spectroscopy, and other methods.

In addition to aggregation assays, other protein degradation methods are discussed in Hochuli (previously cited, and incorporated herein by reference). Additionally, other methods are also possible, which are discussed in the following section.

Example 1: Protein surface mapping:

Environmentally induced degradation of therapeutic proteins will frequently result in a conformational change in the protein. This conformational change becomes particularly problematic when the change in the protein conformation is large enough so as to substantially alter the immunological profile of the protein.

These changes can be assessed by using enzymatic-labeling techniques, which label exposed residues on the surface of biological proteins.

Here the therapeutic protein of interest is labeled or modified by a variety of enzymatic methods. These methods may include protease digestion, posttranslational modification, labeling with a tag that produces a detectable signal, or any method that requires steric access to the protein surface in order to modify the protein structure. The protein may then be fragmented into different peptides by various means (enzymatic digestion, chemical cleavage, etc.), and the amount of label on each fragment, or the presence or absence of digestion products, quantitated by various methods, including peptide mapping, capillary electrophoresis, mass spectrometry, etc.

These labeling experiments are done using both fresh protein, and degraded protein. Those peptide fragments that are associated with degraded proteins may be used as markers to monitor the formation of potentially immunogenic degradation epitopes.

Here, it will be useful to first calibrate these methods on therapeutic proteins with previously characterized immunogenic capability. By compiling a large library of comparative data, an expert system (computerized or otherwise) may be developed that with an ability to correlate changes in therapeutic conformation with development of potential immunogenic activity.

Example 2: Comprehensive mapping of all potentially immunogenic therapeutic protein epitopes:

This technique uses phage display technology, which is reviewed by Petrenko, J Microbiol Methods. 2003 May;53(2):253-62; Coomber, Methods Mol Biol. 2002;178:133-45, and others. Alternatively, ribosome display technology, reviewed by Ling, Comb Chem High Throughput Screen. 2003 Aug;6(5):421-32 or more traditional lymphocyte monoclonal antibody technology may also be used.

Although this comprehensive mapping technique has not been described in previous literature, and thus appears to be a novel aspect of the present invention, it has the potential for creating direct links between protein degradation, the immune response capability of large populations of human subjects, and the development of unwanted immunogenicity.

Here, a phage display or ribosome display library consisting of many different types of antibody genes, or alternatively immune response genes (MHC antigens, Ia antigens' etc.), representative of the various genes distribution in the drug's target population, may be used to construct a "stability epitope map" of the therapeutic protein's temperature or environmentally sensitive regions.

To do this, the phage display or ribosome display library is used to create several libraries of different monoclonal antibodies (or other immune response receptor molecule) with activity against essentially all potential epitopes on the therapeutic protein. These libraries consist of panels of different monoclonal antibodies that bind to different specific regions of interest (epitopes) on the therapeutic protein under investigation. One library might represent the target population's (e.g. the human population that are potential users of the drug) potential capability to mount an immune response against various epitopes on the environmentally stressed therapeutic protein. A second library would represent the target population's potential capability to mount an immune response against the fresh (non environmentally stressed) therapeutic protein. Those monoclonal antibodies (or other immune response receptor molecule), that detect only the new epitopes produced upon thermal environmental stress of the therapeutic protein (anti-degradation specific epitopes) can then be used to form the basis of a "differential immunogenicity risk" assay.

This panel of degradation epitope monoclonal antibodies can then be used to map out the precise details of the therapeutic protein's environmental sensitivity profile. For example, samples of the therapeutic protein may be stressed over comprehensive range of times and temperatures spanning all possible field thermal environments (for example 2 °C, 4

°C, 6 °C ... 38 °C, 40 °C ...48 °C, 50 °C) and over all possible time values up until product expiration (e.g. 1 month, 2 months ... 12 months...18 months). This two dimensional array of stressed therapeutic proteins can then be analyzed using the panel of degradation epitope monoclonal antibodies, and the response curve of time and temperature versus degradation epitope production ascertained.

Next, using historical data based upon comparative studies of therapeutic proteins, which are known to exhibit an acceptable level of immunogenic activity in the general population, a maximum acceptable level of reactivity in the degradation epitope assay is determined. Using this maximum acceptable level, the curve representing the maximum time at each temperature level before the therapeutic protein if interest exceeds the maximum level of reactivity is determined. This is used to produce a time-temperature curve representing the amount of time at any given temperature that the therapeutic protein can exhibit before the risk of unwanted antigenic activity becomes too great.

This data may then be used as input into various types of time-temperature indicator, which then may be affixed to the storage container of the therapeutic protein of interest, forming a unitized device that is continually available to health care workers.

In a modification of this technique, phage display technology may also be used to create a differential epitope map between a natural protein, and a manufactured therapeutic protein, and can be also used to optimize the biochemistry of the manufactured therapeutic protein for maximum immunological stability.

Example 3: Monitoring the formation of protein aggregates. Methods to characterize protein aggregates are well known in the field. One good example is disclosed in the work of DePaolis et. al., "Characterization of erythropoietin dimerization", J Pharm Sci. 1995 Nov;84(11):1280-4. Protein aggregates typically exhibit a large change in molecular weight, which can be monitored by essentially any method sensitive to changes in molecular weight.

Once the relevant time-temperature storage conditions associated with immunogenic risk have been identified, the next step in the present invention is to devise or program suitable time-temperature indicators that can warn users when an unacceptable thermal exposure has occurred. Example 4, shown below, shows how this is done, using the "poster child" of unwanted immunogenic reactions, the recombinant drug "Eprex™", as an example.

Example 4: Use of an electronic time-temperature indicator to monitor the immunological stability of various Erythropoietin drugs.

As previously discussed, certain temperature sensitive forms of Erythropoietin (EPO) have shown a strong correlation with subsequent generation of autoimmune responses against natural erythropoietin. In particular, the bovine serum albumin (BSA) free formulation of Eprex has a history of being particularly problematic. Erythropoietin has a tendency to form aggregates upon storage, and this tendency is accelerated at higher temperatures, as discussed in the DePaolis et. al. article cited earlier. This tendency to form aggregates can be reduced by the proper use of stability enhancers, such as BSA, detergents, and other molecules. The American version of Eprex contained BSA as a stabilizer, and had a good safety track record. The European Union objects to BSA, however, and in 1998, the European version of Eprex was changed to a BSA-free formulation. Within a few months, an unusually large number of red cell aplasia cases were noted in European Eprex users. This disorder, which can result in a complete cessation of red cell production, is caused by an autoimmune reaction against the body's own natural form of erythropoietin.

The reformulated form of Eprex had a higher tendency to form potentially immunogenic aggregates upon exposure to higher temperatures. In an attempt to address this situation, the manufacturer made a point of instructing users that although the product could be safely stored at 4-8 °C for up to 24 months, it should not be kept at room temperature (25 °C) for more than one hour. By contrast, other forms of erythropoietin were capable of being stored for up to 5 days at room temperature (25 °C) without undue chemical

change, aggregation, or denaturation. Thus, in this situation, immunological concerns, coupled with the known physical and chemical changes associated with the reformulated product at various temperatures, forced a major stability derating. Due to the lack of appropriate technology to address the situation, however, this derating could only be addressed by a labeling change.

Although changing the labeling to require more stringent temperature handling precautions was a sensible response to the Eprex immunogenicity problem, this change placed a considerable burden on the users of the product. Without suitable monitoring technology, professional healthcare workers could not easily determine if the product had ever received a cumulative temperature exposure of more than one hour at room temperature. Home users, who typically transport and store the product under less than optimal conditions, were particularly disadvantaged by these stringent handling precautions. Indeed, the revised labeling advised against home use.

Example 4 shows how the electronic time-temperature indicator technology of the copending patent 10/634,297 can assist in managing this type of situation. In this example, the comparative erythropoietin stability data obtained from Anton Haselbeck, "Epoetins: differences and their relevance to immunogenicity", Current Medical Research and Opinions 19(5), p 430-432 (2003), is used to provide input data useful for programming a programmable electronic time-temperature indicator that can warn users when a container of erythropoietin has had a potentially immunologically dangerous thermal history.

A table summarizing Haselbeck's comparative stability data on two different forms of Erythropoietin is shown below:

Table 1: Storage life of two different erythropoietin drugs

	Temperature			
	< 0 °C	4-8 °C (6° C Avg.)	25 °C	Denaturation temp
Eprex (no BSA)	0	24 months	1 hour	53 °C *
NeoRecormon	0	36 months	5 days	53 °C *

* Arakawa et. al., Biosci Biotechnology Biochem 65(6) 1321-1327 (2001)

Eprex (no BSA) is the form of erythropoietin that has a history of generating unwanted immunological reactions. Neorecormon is an alternative form of erythropoietin, produced by a different manufacturer, which has an excellent immunological safety record.

Note that neither form of erythropoietin tolerates freezing, and both have stability data that can be fit by two different Arrhenius plot equations, one covering the range from 1 °C to 25 °C, and the other covering the range from 25 °C to 53 °C. Neither form of erythropoietin tolerates temperatures above 53 °C.

Arrhenius plots: As a brief review, Arrhenius plots are often used to model thermal stability. This type of analysis makes use of the fact that temperature activated reactions, which lie at the heart of thermal stability, are an exponential function of temperature. Thus when the logarithm of product life is plotted versus 1/temperature, the result is typically a straight line, at least over a limited range of temperatures. The slope and intercept of this line can be used to predict the material's stability at various temperatures. Since often, different decay mechanisms are involved at different temperatures, it is helpful to use a series of different Arrhenius equations, each operating over a different temperature domain, as a more accurate way to model a material's stability. This approach is used in this example.

Using Arrhenius log scale techniques, if ln(lifetime) = a + b(1/t) (where t is the temperature in degrees Kelvin), then lifetime = $e^{a} * e^{b/t}$.

Note that the use of Arrhenius plots and equations is not necessarily required, or even preferred. Ideally, a large amount of experimental data is obtained, and an empirical "best fit" curve will be used. However in the absence of large amounts of detailed experimental data, Arrhenius plots and equations have a good track record of accuracy. Thus they will be used in this example.

In this example, the two Erythropoietin drugs are each modeled by four equations, which together cover the temperature range from -20 °C to 70 °C. This range represents the minimum and maximum temperatures that the drugs would ever be likely to encounter in the field. These four equations are:

Equation 1: For storage temperature < 0 °C, storage life = 0 hours.

Equation 2: For storage temperature > 0 °C and <= 25 °C, storage life = $ae^{-b/(T+273)}$, where "a" and "b" are coefficients designed to fit the observed stability of the drug in this temperature range using the 6 °C (which is the average of 4°C and 8 °C) and the 25 °C data points, and "T" represents temperature in degrees centigrade. Here the "273" represents the conversion factor (actually 273.15) needed to convert degrees centigrade into degrees Kelvin, which is needed to properly fit the Arrhenius plot.

Equation 3: For storage temperature > 25 °C and <= denaturation temp., storage life = $ce^{-d/(T+273)}$ where "c" and "d" are coefficients designed to fit the observed stability of the drug between its non-zero storage life at 25 °C, and its zero storage life at the observed denaturation temperature (53 °C), using the 25 °C and 53 °C data points.

Equation 4: For storage temperature > denaturation temperature (53 °C), storage life = 0 hours.

The data from table 1 is fit with an Arrhenius temperature stability model. The equations giving the calculated lifetimes (in hours) of these two drugs as a function of storage temperature (°C) are shown in table 2 below.

Table 2: Lifetime (hours) of Eprex (no BSA) and Neorecormon forms of EPO

	Temperature			
	< 0 °C	1-25 °C	25 - 53 °C	>53 °C
Eprex (no BSA)	0	$4.50*10^{-63}*e^{42802/(t+273)}$	$1.14*10^{-35}*e^{23990/(t+273)}$	0
NeoRecormon	0	$4.93*10^{-33}*e^{23607/(t+273)}$	$8.42*10^{-58}*e^{40617/(t+273)}$	0

The Arrhenius plot calculations show that at the point of maximum stability (1 °C), Eprex has a calculated lifetime of 11,962 days, and Neorecormon has a calculated lifetime of 5,120 days. This paradoxical effect (the higher stability Neorecormon has a lower extrapolated 1 °C shelf-life) is probably not real, and is most likely a mathematical artifact caused by the sharp fall in Eprex stability as a function of temperature between 6 °C and 25 °C. In practice, this artifact would need to be corrected by incorporating additional experimental data into the model. For these calculations, which are primarily concerned with the region between 6 °C and 53 °C, the artifact is minor, and thus the equations will be used as-is.

Using this data, a time-temperature indicator, suitable for warning when the no BSA Eprex has exceeded its recommended thermal profile, can be programmed as originally discussed in copending patent 10/634,297. This process is reviewed below:

To briefly review, copending application 10/634,297 teaches time-temperature monitors that electronically monitor temperature and compute shelf-life, using microprocessors and visual displays that continually compute shelf life using equations of the type:

(Equation 1) $B = F - \sum_{0}^{Time} P(temp)$,

Every few minutes, the device samples the temperature, computes equation 1, and makes an assessment as to if the thermal history has been acceptable or not. Here "B" is the number of points remaining in the units electronic "stability bank", "F" is the initial number of stability points when the product is fresh, and P(temp) is the number of stability points withdrawn from the stability bank each time interval. P(temp) is a function of temperature designed to mimic the product's observed temperature sensitivity. As long as B is greater than zero, the device will display a "+" reading, letting the user know that the drug's stability history has been acceptable. However if B becomes zero or negative, the device will display a "-", indicating that the thermal history is unacceptable.

Using Eprex as an example, the calculations necessary to program the unit to perform equation 1 are shown below.

At the point of maximum stability (1 °C), Eprex has a fresh lifetime "F" of 11,962 days or 287,088 hours. Thus, in this example, assuming that the electronic time-temperature monitor samples the temperature every 6 minutes (1/10 hours), this would be 2,870,879 (6-minute) time units. Since the time-temperature indicators of copending application 10/634,297 use digital arithmetic, to avoid the use of decimal points for the P(temp) values, this stability number "F" will be multiplied by 10 give sufficient resolution to the subsequent integer-based P(temp) values.

Thus, assuming that the temperature is measured every 6 minutes (1/10 hour), and that the minimum P(temp) value is 10, then F = number of time units at the maximum stability temperature = 28,708,793 time units.

So the stability bank "B" for fresh Eprex will have an initial deposit of "F" (28,708,793) units (the equivalent calculations with Neorecormin would result in an initial "F" value of 12,287,123 units). Moreover, if the Eprex is kept at a constant 1 °C temperature, $P(temp_{1C})$ should deduct 10 points per hour from the stability bank "B", and the stability equation (1) would be:

(Equation 2) $B = F - \sum_{0}^{Time} P(temp_{1c})$ thus: $B = 28708793 - \sum_{0}^{Time} 10$ or equivalently:

$B = 28{,}709{,}793 - Time * 10$ Where again, Time is a multiple of 6 minutes (1/10 hour).

To determine the P(temp) values for temperatures above 1 °C, the experimental stability lifetime data is modeled by the best-fit equations from Table 2. As an example, for the region between 1 °C and 25 °C, for Eprex, the stability lifetime calculation is:

(Equation 3) $\text{Stability\_lifetime(hours)} = 4.50\text{x}10^{-63} * e^{42802/(temp+273)}$ where "temp" is the temperature in °C.

To determine the P(temp) values for various temperatures, which is required to program the electronic time-temperature indicators of copending application 10/634,297, it is important to note that at a constant temperature, $temp_c$, equation (1) becomes:

(Equation 4) $B = F - P(temp_c)T$ where "T" is the number of time units.

Now by definition, the stability lifetime is the time "T" when the stability bank "B" first hits zero, so at the stability lifetime limit where B=0, equation (4) becomes:

(Equation 5) $0 = F - P(temp_c)T$ so solving for $P(temp_c)$, then

(Equation 6) $P(temp_c) = \dfrac{F}{T}$

Thus for any given temperature, $P(temp_c)$ is equivalent to the lifetime of the material "F" at the maximum stability temperature, divided by the calculated lifetime of the material at the particular given temperature ($temp_c$).

In this Eprex stability example; the experimental data from table 1, the maximum stability lifetime "F" of 28,708,793, and the best fit stability lifetime from table 2, can be combined with equation (6) to produce a table of P(temp) values, with a temperature granularity of 1 °C, that covers the full temperature range between 1 °C and 25 °C. In a similar manner, the data between 25 °C and 53 °C can be fit by a second set of calculations. The data < 0 °C, and >53 °C, can be fit by a table of constants, where the values of the constants are chosen so as to have the time-temperature unit instantly expire if these temperature values are reached.

These calculations can be used to produce a table of P(temp) values, shown in table 3 below:

Table 3: P(temp) calculations for Eprex and Neorecormon stability between -20 to 70 °C

Temp	Eprex P(temp)	Eprex Lifetime(h)	Neorecormon P(temp)	Neorecorm n Lifetime (h)	Notes
-20	28,708,793	0.1	12,287,123	0.1	
-1	28,708,793	0.1	12,287,123	0.1	
0	28,708,793	0.1	12,287,123	0.1	Freezing
1	10	287087.9	10	122871.2	
2	18	159493.3	14	87765.2	
3	31	92609.0	19	64669.1	
4	54	53164.4	25	49148.5	Low Ref.
5	94	30541.3	34	36138.6	
6	164	17505.4	47	26142.8	Ave. Ref.
7	283	10144.4	63	19503.4	
8	488	5882.9	85	14455.4	High Ref.
9	837	3430.0	115	10684.5	
10	1,429	2009.0	154	7978.7	
15	19,677	145.9	656	1873.0	
20	245,374	11.7	2,653	463.1	
25	2,609,890	1.1	10,231	120.1	Room temp
30	7,177,198	0.4	95,993	12.8	
40	28,708,793	0.1	4,095,708	0.3	
50	28,708,793	0.1	12,287,123	0.1	
53	28,708,793	0.1	12,287,123	0.1	Denaturation
70	28,708,793	0.1	12,287,123	0.1	

To keep the table to a manageable size, suitable for printing, the temperature entries between -2 to -19, 11 to 14, 16 to 19, 21 to 24, and 25 to 29, 31 to 39, 41 to 49, 51-52, and 54 to 69 °C are not shown.

The graphs of comparative Eprex and Neorecormon lifetime as a function of temperature are shown in figure 4. The P(temp) values (number of stability points per 6 minutes or

1/10 hour), which is used to program the time-temperature indicators, are shown in Figure 5.

Time-temperature indicators programmed with this set of P(temp) data can then be included in the no-BSA Eprex packaging, either as an integral part of each container, or as part of a small, multi-container package. Ideally the multi-container is not a large shipping container with hundreds of units, where individual units will be removed and stored at unknown temperatures. Rather, the multi-container should be a small multi-pack, with about 1-20 individual units, so that the individual units will not be removed from the multi-pack, but rather stay with it throughout their storage and use life.

When this configuration is used, the indicator is then able to warn users whenever the thermal-history of the product has exceeded the manufacturer's immunological safety limits. This will help prevent the use of immunologically active degraded material in patients, and thus help reduce the frequency of red cell aplasia.

Figure 6 shows an example of a unitized therapeutic protein storage container (1) constructed according to the teachings of the present invention. This storage container consists of a drug storage compartment (2), which may store the therapeutic protein in a lyophilized state, liquid state, or other state. The storage container also contains an environmental monitor (3), such as the electronic time-temperature indicator of 10/634,297; attached to the protein storage compartment so that the indicator and the storage compartment form a unit. This attachment means may be by a permanent link, or by a detachable link, so that the monitor may be reset and reused once the therapeutic protein has been dispensed. If the monitor is affixed by a detachable link, it may be desirable to use a security seal or other mechanism to detect and discourage tampering with the monitor.

The underside of the storage container is shown in (4). In this example, the monitor has a liquid crystal display (5) that shows if the thermal history of the therapeutic protein is

acceptable from the immunological standpoint (in which case a "+" is shown), or not acceptable (in which case a "-" is shown).

Figure 7 shows an example of a stand-alone time-temperature indicator, suitable for including as part of a multi-pack of multiple storage containers, and designed to comply with relevant Food and Drug Administration (FDA) electronic monitoring requirements. Here, the circuitry is enclosed in case (1) which has a liquid crystal display (2) that displays a "+" symbol if the thermal history of the unit is acceptable (shown), or a "-" if the thermal history is not acceptable (not shown). The unit additionally contains a coin cell battery (3). The front of the unit additionally contains a "data download" button (4), and an infrared (or Radio frequency identification tag - RFID) transmitter (5), so that when the data download button is pressed, relevant statistical information and data validation codes may be transmitted in order to comply with FDA electronic records requirements. The back of the unit, shown in (6) exposes the unit's temperature sensor to the environment inside the multi pack through a temperature sensor mounted on the case surface (7).

Figure 8 shows an example of a multi-pack (1) of pharmaceutical vials (2), containing an electronic time-temperature indicator similar to that of Figure 7 at one end (3).